

CLAIMS

1. A method for identifying a microorganism having a reduced adaptation to a particular environment comprising the steps of:
- 5 (1) providing a plurality of microorganisms each of which is independently mutated by the insertional inactivation of a gene with a nucleic acid comprising a unique marker sequence so that each mutant contains a different marker sequence, or clones of the said microorganism;
- (2) providing individually a stored sample of each mutant
10 produced by step (1) and providing individually stored nucleic acid comprising the unique marker sequence from each individual mutant;
- (3) introducing a plurality of mutants produced by step (1) into the said particular environment and allowing those microorganisms which are able to do so to grow in the said environment;
- 15 (4) retrieving microorganisms from the said environment or a selected part thereof and isolating the nucleic acid from the retrieved microorganisms;
- (5) comparing any marker sequences in the nucleic acid isolated
20 as in step (4) to the unique marker sequence of each individual mutant stored as in step (2); and
- (6) selecting an individual mutant which does not contain any of the marker sequences as isolated in step (4).
2. A method according to Claim 1 wherein the plurality of
25 microorganisms as defined in step (1) is produced from a plurality of microorganisms, each of which comprises a nucleic acid comprising a unique marker sequence, by changing their condition from a first given condition to a second given condition wherein (a) in the first given condition the said nucleic acid comprising a unique marker is maintained episomally
30 and (b) in the second given condition the said nucleic acid comprising a

09744602 111600

unique marker sequence ~~insertionally~~ inactivates a gene.

3. A method according to Claims 1 or 2 further comprising the steps:
(1A) removing auxotrophs from the plurality of mutants produced
5 in step (1); or
(6A) determining whether the mutant selected in step (6) is an
auxotroph; or
both (1A) and (6A).
- 10 4. A method of identifying a gene which allows a microorganism to
adapt to a particular environment, the method comprising the method of any
one of Claims 1 to 3 followed by the step:
(7) isolating the insertionally-inactivated gene from the individual
mutant selected in step (6).
- 15 5. A method according to Claim 4 further comprising the step:
(8) isolating from a wild-type microorganism the corresponding
wild-type gene using the insertionally-inactivated gene isolated in step (7)
as a probe.
- 20 6. A method according to any one of Claims 1 to 5 wherein the
particular environment is a differentiated multicellular organism.
- 25 7. A method according to Claim 6 wherein the multicellular organism
is a plant.
8. A method according to Claim 6 wherein the multicellular organism
is a non-human animal.
- 30 9. A method according to Claim 8 wherein the animal is a mouse, rat,

09714602 11600

rabbit, dog or monkey.

10. A method according to Claim 9 wherein the animal is a mouse.
- 5 11. A method according to any one of Claims 6 to 10 wherein in step (4) the microorganisms are retrieved from the said environment at a site remote from the site of introduction in step (3).
12. A method according to any one of Claims 8 to 10 wherein in step (3)
10 the microorganism is introduced orally or intraperitoneally.
13. A method according to Claim 12 when dependent on Claims 8 or 9 wherein in step (4) the microorganisms are retrieved from the spleen.
- 15 14. A method according to any one of the preceding claims wherein the microorganism is a bacterium.
15. A method according to any one of Claims 1 to 13 wherein the
microorganism is a fungus.
- 20 16. A method according to Claim 7 wherein the microorganism is a bacterium pathogenic to plants.
17. A method according to Claim 7 wherein the microorganism is a
25 fungus pathogenic to plants.
18. A method according to any one of Claims 8 to 10 wherein the microorganism is a bacterium pathogenic to animals.
- 30 19. A method according to any one of Claims 8 to 10 wherein the

09714602 111600

microorganism is a fungus pathogenic to animals.

20. A method according to Claim 18 wherein the bacterium is any one of *Bordetella pertussis*, *Campylobacter jejuni*, *Clostridium botulinum*,
5 *Escherichia coli*, *Haemophilus ducreyi*, *Haemophilus influenzae*,
Helicobacter pylori, *Klebsiella pneumoniae*, *Legionella pneumophila*,
Listeria spp., *Neisseria gonorrhoeae*, *Neisseria meningitidis*, *Pseudomonas*
spp., *Salmonella* spp., *Shigella* spp., *Staphylococcus aureus*, *Streptococcus*
pyogenes, *Streptococcus pneumoniae*, *Vibrio* spp., and *Yersinia pestis*.
10
21. A method according to Claim 19 wherein the fungus is any one of
Aspergillus spp., *Cryptococcus neoformans* and *Histoplasma capsulatum*.
22. A method according to any one of the preceding claims wherein in
15 step (1) the gene is insertionally inactivated using a transposon or
transposon like element or other DNA sequence carrying a unique marker
sequence.
23. A method according to any one of the preceding claims wherein in
20 step (1) each different marker sequence is flanked on either side by
sequences common to each said nucleic acid.
24. A method according to Claim 23 wherein in step (2) the nucleic acid
comprising the unique marker is isolated using DNA amplification
25 techniques and oligonucleotide primers which hybridise to the said common
sequences.
25. A method according to Claim 23 or 24 wherein in step (4) the
nucleic acid comprising a plurality of said marker sequences is isolated
30 using DNA amplification techniques and oligonucleotide primers which

09714602 111600

hybridise to the said common sequences.

26. A microorganism obtained using the method of any one of the preceding claims.

5

27. A microorganism comprising a mutation in a gene identified using the method of Claim 5.

10

28. A microorganism obtained according to Claim 26, when dependent on Claim 8, or Claim 27 for use in a vaccine.

29. A vaccine comprising a microorganism according to Claim 26, when dependent on Claim 8, or Claim 27 and a pharmaceutically-acceptable carrier.

15

30. A gene obtained using the method of Claims 4 or 5.

20

31. A gene according to Claim 30 which is isolated from the *Salmonella typhimurium* genome and hybridises to the sequence shown in Figure 5 under stringent conditions.

25

32. A gene according to Claim 30 which is isolated from the *Salmonella typhimurium* genome and hybridises to a sequence shown in Figure 6 under stringent conditions.

33. A polypeptide encoded by a gene according to any one of Claims 30 to 32.

30

34. A method of identifying a compound which reduces the ability of a microorganism to adapt to a particular environment comprising the step of

09714602.11600

selecting a compound which interferes with the function of a gene according to any one of Claims 30 to 32 or a polypeptide according to Claim 33.

35. A compound identifiable by the method of Claim 34.

36. A compound according to Claim 35 wherein the particular environment is a host organism.

37. A compound according to Claim 36 wherein the host organism is a plant.

38. A compound according to Claim 36 wherein the host organism is an animal.

39. Use of a compound according to any one of Claim 36 to Claim 38 for treating infection of said host organism with said microorganism.

40. A molecule which selectively interacts with, and substantially inhibits the function of, a gene according to any one of Claims 30 to 32 or a nucleic acid product thereof.

41. A molecule according to Claim 40 which is an antisense nucleic acid or nucleic acid derivative.

42. A molecule according to Claim 40 or 41 which is an antisense oligonucleotide.

43. A molecule according to any one of Claims 40 to 42 for use in medicine.

09714602-11600

44. A method of treating a host which has, or is susceptible to, an infection with a microorganism, the method comprising administering an effective amount of a molecule or compound according to Claim 36 or 40 wherein said gene is present in said microorganism, or a close relative of said microorganism.

45. A pharmaceutical composition comprising a molecule or compound according to Claim 38 or 40 and a pharmaceutically acceptable carrier.

46. The VGC2 DNA of *Salmonella typhimurium* or a part thereof, or a variant of said DNA or a variant of a part thereof.

47. A mutant bacterium wherein if the bacterium normally contains a gene that is the same as or equivalent to a gene in VGC2, said gene is mutated or absent in said mutant bacterium.

48. A method of making a bacterium according to Claim 47.

49. Use of a mutant bacterium according to Claim 47 in a vaccine.

50. A pharmaceutical composition comprising a bacterium according to Claim 47 and a pharmaceutically acceptable carrier.

51. A polypeptide encoded by VGC2 DNA of *Salmonella typhimurium* or a part thereof, or a variant of said polypeptide or a variant of a part thereof.

52. A method of identifying a compound which reduces the ability of a bacterium to infect or cause disease in a host comprising the step of selecting a compound which interferes with the function of a gene in VGC2

09714602-11600

claim 46 or a polypeptide
bound identifiable by
molecule which selectively
binds to a gene in VGC2
cell.
molecule or compound a
feature or combi

[Signature]

5

10

add 25